

III. Specification Amendments

Please replace the paragraph beginning on page 13, line 35 through page 14, line 7 with the following paragraph:

A 10 mm x 10 cm (8.0 ml) ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-M column (TosoHaas, Montgomeryville, PA) was equilibrated at 2 ml/min in 15 mM TEAP, pH 7.2. A 27 ml aliquot of GHA (2.6 mg/ml, dissolved in 50 mM Tris, pH 7.2, 200 mM NaCl) was loaded onto the equilibrated CG71-M column and then washed with equilibration buffer. The column was eluted stepwise with hexanediol beginning with 32.5% hexanediol in 15 mM TEAP, pH 7.2 then with 49% hexanediol in 15 mM TEAP, pH 7.2 and 65% hexanediol in 15 mM TEAP, pH 7.2. The eluant was monitored by absorbance at 280 nm and collected fractions were analyzed by SDS-PAGE and RP-HPLC.

Please replace the paragraph beginning on page 14, line 8 through page 14, line 15 with the following paragraph:

The chromatography results from the 8.0 ml ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-M column are shown in Figure 4. GHA was eluted by a step gradient of 32.5% hexanediol in TEAP, pH 7.2 with a yield of 98%. Figure 5 shows SDS-PAGE analysis of the eluted protein. As can be seen in Figure 5, lane 10, the eluted protein consists of primarily GHA along with some contaminants. Fractions 10 through 14 were pooled and analyzed for protein content by C-4 RP-HPLC, which is shown in Figure 6. The pool volume was

20 ml at 3.43 mg/ml, which translates to 68.6 mg total GHA. The amount of GHA loaded onto the column was about 69 mg.

Please replace the paragraph beginning on page 15, line 7 through page 15, line 17 with the following paragraph:

Three columns containing ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-M (TosoHaas, Montgomeryville, PA), ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-C and ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG300-M resin, (TosoHaas, Montgomeryville, PA) respectively were equilibrated in 15 mM TEAP, pH 7.2. Each column was loaded with 40 ml of diluted top phase material (prepared by two-phase extraction, using the procedure of Hayenga et al., United States Patent Application Serial No. 09/307,549) which contained about 0.5 mg/ml GHA. The columns were loaded at 10 mg/ml. After loading, each column was washed with 15 mM TEAP, pH 7.2. was used to elute GHA from the columns (about 20 column volumes of solvent were used). Effluent was monitored by absorbance at 280 nm and appropriate fractions were collected. Fractions were analyzed by C-4 RP-HPLC and SDS-PAGE.

Please replace the paragraph beginning on page 15, line 18 through page 15 line 24 with the following paragraph:

It was decided to use the method of the present invention as a replacement for a

~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG1000 column, which is a styrene divinylbenzene based reverse phase resin that was previously used to purify GHA from diluted top phase obtained using the procedure of Hayenga *et al.*, United States Patent Application Serial No. 09/307,549. Acetonitrile was typically used to elute GHA from ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG1000 column, which significantly increased cost associated with the process because of handling requirements and expensive disposal.

Please replace the paragraph beginning on page 15, line 25 through page 15, line 31 with the following paragraph:

Thus, ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-C, ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-M methacrylate resins and ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG300-M styrene divinylbenzene resin loaded as described above were eluted using a 1, 6 hexanediol gradient specified above. Figures 10, 11 and 12 show the elution profiles generated on each resin, respectively. Each chromatogram illustrates that 1, 6 hexanediol can elute protein from each resin. Figure 13 illustrates SDS-PAGE analysis for CG71C and CG71M elution pools, while Figures 15 and 16 are C-4 RP-HPLC chromatograms for CG71C and CG71M elution pools, respectively.

Please replace the paragraph beginning on page 15, line 32 through page 16, line 3 with the following paragraph:

Fractions 11 through 18 (24 ml) comprised the pooled fractions for the ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-C experiment (84% yield of GHA). The remaining material was found in the void volume, which suggests that the binding capacity of this resin for is about 8 mg/ml of GHA. Fractions 10 through 16 (21 ml) were pooled for the ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-M experiment (103% yield of GHA). The loading for this column, based upon GHA, is 10 mg/ml. No GHA was detected in the ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG71-M void volume. The ~~data~~ data is summarized in Table 1, shown below.

Please replace the paragraph on page 16, line 20 through page 16, line 24 with the following paragraph:

The ~~Amberchrom~~ AMBERCHROM (a commercially available resin) CG3000-M experiment and the resulting C-4 RP-HPLC and SDS-PAGE analysis (Figures 17 and 14, respectively) shows that some GHA is eluted from this column by 1, 6 hexanediol. However, the profile (Figure 11) shows that elution is diffuse and trials beyond the end of the gradient. Thus, under these conditions, 50% 1, 6 hexanediol fails to completely elute GHA from the resin.

Please replace the paragraph on page 16, line 27 through page 16, line 34 with the following

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paragraph:

Since ~~Amberechrom~~ AMBERCHROM (a commercially available resin) CG71-M resin provided favorable results in the preliminary experiments described in Example 4, this resin was selected for the scale-up experiment. A 235 ml (5cm x 12 cm) ~~Amberechrom~~ AMBERCHROM (a commercially available resin) CG71-M column was poured and equilibrated in 50 mM Tris HCl, pH 7.2. Four liters of 2x diluted top phase, obtained by the procedure of Hayenga et al., United States Patent Application Serial No. 09/307,549, was loaded on the column at 20 ml/min and eluted with a step gradient of 50% 1, 6, hexanediol in 50 mM Tris HCl, pH 7.2. The Column was monitored by absorbance a 280 nm and fractions were collected and analyzed by C-4 RP-HPLC and SDS-PAGE.

Please replace the paragraph beginning on page 16, line 35 through page 17, line 9 with the following paragraph:

The chromatogram for the 235 ml ~~Amberechrom~~ AMBERCHROM (a commercially available resin) CG71-M experiment is shown in Figure 18. The elution profile shows one peak, which is consistent with a step elution at 50% 1, 6 hexanediol. The column load for this experiment was 8.3 mg/ml (about 2000 mg on 235 ml of resin). The C-4 RP-HPLC Chromatogram (Figure 19a) for the void volume shows no GHA. However, the yield of GHA was only 67%. Fractions 6-12 constitute the main peak, which accounts for 47% of the material eluted. Fractions 1 to 5 and 13 to 20 make up the balance. The C-4 RP-HPLC chromatogram (Figure 19b) for the pooled fractions showed essentially pure GHA. Later experiments

demonstrated that a linear gradient from 0% to 50% 1, 6 hexanediol in 50 mM Tris HCl, pH 7.2 provided superior yields of GHA, which were generally between 80 and 95%.

Please replace the paragraph beginning on page 17, line 12 through page 17, line 21 with the following paragraph:

A 100 L (100cm x 12 cm) ~~Amberechrom~~ AMBERCHROM (a commercially available resin) CG71-M column was equilibrated with 3 column volumes of 50mM TRIS, pH 7.2, at 150 cm/hr. Diluted top phase (about 1000 L) containing GHA, obtained from the procedure Hayenga et al., United State Patent Application Serial No. 09/307,549 was loaded onto the column at 7.9 L/min. About 10 g of GHA per L of resin was loaded on the column. The column was then washed with 2 column volumes of 50 mM Tris, pH 7.2 at 60 cm/hr and 3 column volumes of 50 mM Tris, pH 7.2 at 120 cm/hr. GHA was eluted with a 20 column volume gradient from 0 to 50% 1, 6 hexanediol. Buffer A is 50 mM Tris, pH 7.2 (10 column volumes) and buffer B is 50% (w/w) 1, 6 hexanediol, 50 mM Tris, pH 7.2 (10 column volumes). Elution of GHA was monitored by absorbance at 280 nm.